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ISOLATION OF 11-oxoTETRODOTOXIN FROM THE PUFFER AROTHRON NIGROPUNCTATUS

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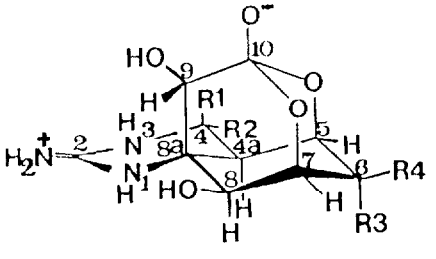
Summary: A novel tetrodotoxin analog was isolated from the puffer Arothron nigropunctatus and identified as 11-oxotetrodotoxin hydrate on the basis of NMR and chemical transformation studies.

Tetrodotoxin (TTX, **1**) has been the subject of investigations for its extremely potent neurotoxicity and unique chemical as well as pharmacological properties. In pursuit of our studies on its metabolic pathway[1] we first isolated 4-epiTTX(**2**), 4,9-anhydroTTX, tetrodonic acid[2] and recently 6-epiTTX (**3**)[3,4],11-deoxyTTX (**4**)[3,4] and 11-norTTX-6(R)-ol (**5**)[4] from puffers and newts. We report here the isolation and structural assignment of 11-oxoTTX from a southern puffer.

Seven specimens of Arothron nigropunctatus (1.2 kg) collected in Micronesia in July 1987 were extracted with hot 0.1% HOAc/H_2O. The extracts were chromatographed successively on columns of charcoal (aqueous EtOH containing 1% HOAc), Bio-Gel P-2, Bio-Rex 70, Hitachi cation exchange gels 3011c and 3013c, and TSK Gel G1000 PW; all the columns except for the charcoal were eluted with 0.05N HOAc. Separation of TTX analogs was monitored by the fluorometric HPLC designed for microdetection of TTX and its congeners[5,6] and by TLC (silica gel 60 with pyridine-EtOAc-HOAc-H_2O, 10:5:2:3).

In addition to TTX, 4-epiTTX and 4,9-anhydroTTX, new analogs[3,4] 6-epiTTX, 11-deoxyTTX and 11-norTTX-6(R)-ol, were isolated from the puffers and identified by comparing chromatographic properties on TLC and fluorometric HPLC. A new analog (3 mg) was eluted before **1**[7] and isolated as a colorless amorphous solid. High resolution FAB-MS recorded on a JEOL JMS DX-303HF spectrometer indicated a probable formula, $C_{11}H_{17}N_3O_9$ (MH^+, m/z 336.1025; found, m/z 336.0965), that was larger than **1** by one oxygen. ^1H NMR spectra

	R1	R2	R3	R4
1 TTX	H	OH	OH	CH_2OH
2 4-epiTTX	OH	H	OH	CH_2OH
3 6-epiTTX	H	OH	CH_2OH	OH
4 11-deoxyTTX	H	OH	OH	CH_3
5 11-norTTX-6(R)-ol	H	OH	H	OH
6 11-oxoTTX	H	OH	OH	$CH(OH)_2$



(JEOL, GSX-400) of **6** in 4% CD_3COOD/D_2O showed coupled signals at **δ** 2.31 (br.d 9.5 Hz) and 5.51 (d 9.5 Hz) corresponding to the characteristic signals of H-4a and H-4 in **1**. Four oxymethine signals at **δ** 3.98, 4.19. 4.27 and 4.37, were assignable to H-9, H-7, H-8 and H-5 of a TTX skeleton on the basis of spin decouplings and NOE experiments. The signals due to CH_2-11 of **1**, however, were absent, and a singlet newly appeared at **δ** 5.74. Positive NOE's (small percentages, respectively) on H-5 and H-7 upon irradiation at **δ** 5.74 supported the finding that the new signal was due to a proton on C11. Its chemical shift, typical for an acetal methine, as well as the formula deduced by HR-FABMS evidenced that C11 was oxidized to aldehyde, which took a hydrate form under the conditions of NMR and FAB-MS measurements. The structure was further supported by reduction of **6** with $NaBH_3CN$[8]; the product was indistinguishable from **1** on the fluorometric HPLC[6,7] and gave an MH^+ ion at m/z 320 in a FAB-MS as dose **1**. The configuration of C11 was assigned to be equatorial, because HPLC analyses[6] of the reduction products did not reveal the presence of 6-epiTTX, an expected product from an epimer at C6 of **6**. The absence of NOEs on H-4a and H-8 upon irradiation at H-11 also supported the equatorial assignment of C11. Based on the above data, we assigned the structure of the new analog to **6**. The isolation and structural assignment of this analog was thus successfully achieved for the first time. This finding clearly shows that a series of oxidation takes place at C11 in puffers. A search for an analog oxidized to a carboxylic acid at C11, a missing link between **5** and **6**, is under way.

Reference and Notes

1)(a) T. Yasumoto, H. Nagai, D. Yasumura, T. Michishita, A. Endo, M. Yotsu and Y. Kotaki, Ann. N.Y. Acad. Sci, **479,** 44 (1986). (b) T. Yasumoto, D. Yasumura, M. Yotsu, T. Michishita, A. Endo, Y. Kotaki, Agric. Biol. Chem., **50,** 793 (1986). (c) M. Yotsu, T. Yamazaki, Y. Meguro, A. Endo, M. Murata, H. Naoki and T. Yasumoto, Toxicon, **25,** 225 (1987).

2) M. Nakamura and T. Yasumoto, Toxicon, **23,** 271 (1985).

3) T. Yasumoto, M. Yotsu, M. Murata and H. Naoki, J. Am. Chem. Soc., **110,** 2344 (1988).

4) A. Endo, S.S. Khora, M.Murata, H.Naoki and T. Yasumoto, Tetrahedron Lett., **29** (33), 4127 (1988).

5) T. Yasumoto and T. Michishita, Agric. Biol. Chem., **49,** 3077 (1985).

6) M. Yotsu, A. Endo and T. Yasumoto, Agric. Biol. Chem., **53,** 895 (1989).

7) Rf. values on TLC: 11-oxoTTX 0.42, TTX 0.67; Retention volume on the fluorometric HPLC[6]: 11-oxoTTX 5.67 ml, TTX 5.96 ml; Minimum lethal dose (mice, ip.) 120 μg/kg (acetate salt).

8) The analog (60 nM) was treated with $NaBH_3CN$ (350 nM) in 0.5 M HOAc (20 μl) at room temperature for 15 h.

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